

2071-Pos Board B57**Solid-State NMR Study on the Conductance Mechanism and Acid Activation of M2 Proton Channel**

Thach Can, Yimin Miao, Mukesh Sharma, Sorin Luca, Huajun Qin, Ivan Hung, Milton Truong, David Busath, Huan-Xiang Zhou, Timothy Cross. M2 is a 97-residue, single-pass membrane protein which functions in a tetrameric state. The tetramer forms a proton channel which has been proven to be a drug target for *Influenza A* virus (H3N2 and H1N1). However, the M2 protein of this virus has recently mutated so that the anti-flu drugs, amantadine and rimantadine are no longer effective; consequently there is increased urgency to define the structure and functional mechanism of this protein as an aid for drug development. Even though it has long been believed that M2 protein is a proton channel with a high selectivity, most functional assays find a low conductance rate which resembles the activity of a transporter. An atomistic mechanism has recently been proposed for the proton transport involving the His37 and Trp41 residues in this tetramer [1]. Here we present magic angle spinning solid state NMR spectroscopic data of the M2(22-62) construct and the full-length protein that bear on this mechanistic model and on the pH-induced structural changes. Solid state NMR provides an opportunity to characterize the protein in a native-like lipid bilayer environment. Recently, a comparison of M2 transmembrane domain structures obtained from detergent and lipid bilayer environments has demonstrated the importance of using good models of the membrane environment [2].

[1] M. Sharma, M. Yi, H. Dong, H. Qin, E. Peterson, D. D. Busath, H.-X. Zhou, and T. A. Cross (2010). Insight into the mechanism of the influenza A proton channel from a structure in a lipid bilayer. *Science* (in press).

[2] T. A. Cross, M. Sharma, M. Yi, and H.-X. Zhou (2010). Influence of solubilizing environments on membrane protein structures. *Trends Biochem. Sci.* (in press).

2072-Pos Board B58**Structure and Topology of Phospholamban Bound to Ca^{2+} -ATPase in Lipid Bilayers Studied by Hybrid NMR Methods**

Martin Gustavsson, Nathaniel J. Traaseth, Raffaello Verardi, Gianluigi Veglia.

Phospholamban (PLN) is an integral membrane protein that regulates Sarco-plasmic Reticulum Ca^{2+} -ATPase (SERCA) in cardiomyocytes. Its structure and topology have been solved by a hybrid method of solution and solid-state NMR in detergent micelles and lipid bilayers. The combination of NMR restraints and explicit MD simulations in DOPC clearly reveals PLN's architecture: 1) a regulatory cytoplasmic helix (domain Ia) that is absorbed on the bilayer surface, 2) a hydrophilic helix (domain Ib) that hydrogen bonds with lipid head groups, and 3) a transmembrane helix (domain II) that spans the most hydrophobic part of the membrane. To build on the hybrid method and fully elucidate the structure and topology of PLN when bound to SERCA, we performed magic angle spinning (MAS) experiments combined with oriented solid-state NMR in lipid bilayers under fully functional conditions. This method eliminates the need for solution NMR in detergent micelles, which can distort the structures of membrane proteins. Since high degeneracy of hydrophobic amino acids in PLN (Leu, Val, Ile, Ala) leads to severe spectral overlap for uniformly ^{13}C , ^{15}N -labeled samples, we used selective labeling to enable complete backbone and side chain assignments. The chemical shifts measured by MAS experiments show that the secondary structure of PLN is unchanged upon binding SERCA (i.e., domain II is still helical). However, we observed methyl group chemical shift perturbations (e.g., Ile 38-Cg2, Cd1) that are consistent with the SERCA-interacting face of PLN that was previously proposed from cross-linking data. Oriented NMR experiments showed that the domain II tilt angle of SERCA-bound PLN is conserved between magnetically aligned DMPC/D6PC bicelles and mechanically aligned DOPC/DOPE bilayers. The use of our hybrid approach to study the PLN/SERCA complex (~110 kDa) demonstrates its applicability to other membrane protein complexes.

2073-Pos Board B59**Tuning the Structural Coupling Between the Transmembrane and Cytoplasmic Domains of Phospholamban to Control SERCA Inhibition**

Kim N. Ha, Martin Gustavsson, Raffaello Verardi, Gianluigi Veglia.

Phospholamban (PLN) is the endogenous inhibitor of the sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA), the integral membrane enzyme responsible for 70% of the Ca^{2+} shuttling into the SR, inducing cardiac muscle relaxation in humans. Dysfunctions in SERCA:PLN interactions have been implicated as having a critical role in cardiac disease. Based on the success of the Chien Group with S16E PLN, a pseudo-phosphorylated PLN species which halted the progression of heart failure in animal models, we wish to fur-

ther develop PLN species to improve SERCA function by rationally designing mutations based on the structural and biophysical data available on the system. The present study seeks to introduce single and multiple glycine mutations in the loop domain of PLN in order to tune the structural coupling between the cytoplasmic and transmembrane helix, and ascertain the effects on SERCA function. These studies further develop the model by which the control of enzyme function is performed by altering the structural dynamics of a small inhibitor can then be translated to other membrane enzymes, such as the Na/K-ATPase.

2074-Pos Board B60**Mycobacterium Tuberculosis Protein Rv1861: Structural Insights into GTP Hydrolysis in a Native-Like Membrane Environment**

Dylan T. Murray, Nabanita Das, Timothy A. Cross.

1/3 of the world's population is infected with *Mycobacterium tuberculosis* 10% of whom will become sick from the bacilli. Multidrug resistant strains resistant to the leading tuberculosis antibiotics, isoniazid and rifampicin have emerged necessitating new treatments. Integral membrane proteins are an excellent source of novel drug targets. Structural biology can provide rich information regarding the influence of lead compounds on protein function. Solid state nuclear magnetic resonance is increasingly being used to understand membrane protein structure and dynamics in lipid membrane environments. The structural information can be accompanied with other biophysical techniques to make conclusions regarding membrane protein function and the influence of regulatory compounds. Rv1861 is an integral membrane protein from *Mycobacterium tuberculosis*. It contains the signature GTP binding domain motif AXXXXGKT near the N-terminus of the protein and is predicted to contain three transmembrane α helices from hydrophobicity analysis.

Oriented sample solid state magnetic resonance accurately measures peptide plane orientations through PISEMA experiments on protein in liquid crystalline lipid bilayers aligned between glass slides. The structural data can be correlated to functional information from experiments such as isothermal titration calorimetry on the protein in liposomes allowing protein structure and function to be understood in a native-like environment. Here we present the initial structural characterization of the Rv1861 protein in lipid bilayers. PISEMA experiments on uniformly and amino acid type specifically labeled samples are used to determine helix orientation. Light scattering, size exclusion chromatography and electrophoresis experiments on the protein in micellar environments will provide the oligomeric state and complement the secondary structure characterization. The data provide a firm foundation for further structure determination and functional characterization of the protein in a native-like environment.

2075-Pos Board B61**Understanding the Potential Interaction of Transmembrane Helices from MgtC and MgtR**

Frantz L. Jean-Francois, Timothy A. Cross.

Beyond the challenge consisting of solving a membrane protein structure, Solid state NMR is the most powerful technique to decipher protein dynamics within the membrane hydrophobic core. Recent results have converged to highlight the role of hydrophobic peptides that form a novel class of active molecules in *Escherichia coli* and *Salmonella enteric serovar Typhimurium*. These peptides are apparently able to interact with membrane proteins leading to their inactivation through an unfolding process. Understanding the molecular mechanism of these peptide-protein interactions would offer great opportunities in pharmacology and drug design.

MgtR, a highly hydrophobic 30 amino acids peptide, which is expressed in *Salmonella typhimurium*, is one of these active molecules. It has been shown that within the macrophage, the over expression of MgtR leads to a decrease of *Salmonella* replication rate. This process was shown to involve an interaction between MgtR and a membrane protein of *Salmollena* (MgtC) which is required for the bacteria survival at low magnesium concentration. Throughout the literature, MgtC has proven to be one of the key factors in *Mycobacterium tuberculosis* latent state. Relying on the sequence homology between *Salmonella typhimurium* MgtC and *Mycobacterium tuberculosis* MgtC, the interaction between MgtR from *Salmonella typhimurium* and the transmembrane domain #4 of *Mycobacterium tuberculosis* MgtC will be studied.

Structural studies using solid state NMR methods such as CP-MAS and PI SEMA on oriented samples have been performed. Several ^{15}N labeled peptides were produced by chemical synthesis and biological expression prior reconstitution into model membranes. These NMR studies allowed us to determine theses peptides topology and their backbone structure within membranes.

Interaction studies between these two peptides are currently in progress and will allow us to define the complex structure as well as the amino acids involved in the interaction.

2076-Pos Board B62

Structural Studies of Super-Mini-B: Role of the N-Terminal Insertion Sequence of Surfactant Protein B

Mahzad Sharifmadian, Alan J. Waring, Frans J. Walther, Rober H. Notter, Valerie Booth.

Lung surfactant is a mixture of phospholipids and proteins that is critical for breathing. The hydrophobic surfactant protein B (SP-B) is essential for the function of lung surfactant. Super Mini-B is a 41-residue peptide with internal disulfide bonds that contains the N-terminal 7-residue insertion sequence and the N- and C-terminal helices of SP-B. It has previously been shown to retain the similar activity to full-length SP-B as measured by dynamic compliance and arterial oxygenation in rat studies. Interestingly, Super-Mini-B performs better than peptides which lack the N-terminal insertion sequence (e.g. Mini-B). We have used circular dichroism and solution and solid state NMR to study Super-Mini-B's structure and lipid interactions in various environments, including anionic micelles. Comparison of the results for Mini-B and Super Mini-B help to unveil the contribution of the 7-residue insertion sequence to the function of SP-B.

2077-Pos Board B63

Investigating the Relationship Between Physical Properties of Detergents and Membrane Protein Structure Determination

Ryan Oliver, Ryan Lo, Justin Kim, Linda Columbus.

Membrane proteins comprise only 1.8% of the atomic-resolution structures deposited in the RSB Protein Data Bank (<http://pdhtm.enzim.hu>). One bottleneck contributing to the under-representation of membrane protein structures is the requirement of a membrane mimic, such as a detergent micelle, to solubilize the membrane protein and form a stable protein-detergent complex (PDC). The mimic that leads to a structure is typically determined by empirically and exhaustively screening commercially available detergents. Understanding the relationship between detergent micelle physical properties and the stability, fold, and function of membrane proteins in PDC's reduces the extent of empirical screening required. Additionally, many structures are determined in detergent mixtures and with additives that affect the micelle properties and the protein-detergent complex. The aim of this study is to investigate micelle physical properties, both pure and mixed, and with additives commonly used in structural biology such as polyethylene glycols and cholesterol. In particular, we will present the thermodynamic properties of micelle formation (using isothermal titration calorimetry) and micelle size and shape (using small angle x-ray scattering) of binary detergent mixtures, as well as with additives. We will also present preliminary NMR data of membrane proteins in the characterized micelles in order to investigate trends of detergent properties that correlate with stabilization of a protein fold. These results provide a foundation for understanding the role of detergents in membrane protein structure determination.

2078-Pos Board B64

NMR Backbone Assignment of OpaI: A Mediator of Host:Neisseria Interactions

Daniel A. Fox, Linda Columbus.

Opa (opacity associated) proteins from *Neisseria gonorrhoeae* (NG) and *Neisseria meningitidis* (NM) induce phagocytosis of the bacterium by non-phagocytic cells such as epithelial cells. There are eleven NG and four NM Opa alleles that undergo phase-variation expression to the outer membrane. The protein products are nearly identical in sequence, but vary in three extracellular loop regions that determine the host receptor specificity. Opa proteins bind to carcinoembryonic antigen-like cellular adhesion molecules (CEACAMs) or to heparansulfate proteoglycan receptors (HSPGs), thus subdividing Opa proteins into two classes, OpaCEA and OpaHS, respectively. Mutational and chimeric experiments have not revealed the sequence determinants of the hypervariable regions that are responsible for receptor recognition. The goal of this study is to investigate the structure, dynamics, and receptor interactions of OpaI, a 27 kDa, 238 amino acid OpaCEA from NG MS11. OpaI was over-expressed in *E. coli*, purified, and refolded in dodecylphosphocholine. Two-dimensional ¹⁵N, 1H-TROSY spectra, CD, and SDS-PAGE gel mobility of the protein-detergent complex indicate that the protein is folded and well-suited for NMR studies. Using TROSY-based pulse sequences, methyl labeling, and specific amino acid labeling, a suitable backbone assignment was achieved and an initial low-resolution structural calculation with H-bond and NOE-derived restraints is presented.

2079-Pos Board B65

Investigation of the Neisseria Gonorrhoeae Opacity Associated Membrane Protein A Structure, Dynamics, and Host-Receptor Interactions

Ryan H. Lo.

Opacity associated (Opa) outer membrane proteins from the bacteria *Neisseria meningitidis* and *Neisseria gonorrhoeae* bind to human host cell receptors, which triggers phagocytosis of the bacteria. Opa proteins are classified into two groups (Opa_{CEA} and Opa_{HS}) based on host receptor specificity. Opa_{CEA} interacts with carcinoembryonic antigen-related cell adhesion molecules (CEACAM) and Opa_{HS} binds heparansulfate proteoglycan (HSPG) receptors. Two hypervariable extracellular loops are proposed to determine receptor specificity; however, the molecular determinants of the interaction and host receptor specificity are unknown. The aim of this study is to determine the structure of OpaA (an Opa_{HS} from *N. gonorrhoeae* MS11) using NMR, and to map the specific loop interactions of OpaA with heparin, a competitive inhibitor of the Opa_{HS} and HSPG interaction. The expression, purification, and folding of OpaA will be presented as well as the progress towards the NMR backbone assignment. In addition, the feasibility of using chemical shift perturbation to map the OpaA - heparin interaction will be explored. These investigations of the binding specificity of OpaA to heparin will further the understanding of the pathogenesis of *Neisseria*, which will in turn increase our understanding of an attractive function for receptor-targeted therapeutics.

2080-Pos Board B66

NMR Structural Studies of a Two-Transmembrane Helix Segment of the Na⁺/H⁺ Exchanger Isoform 1

Brian L. Lee, Claudia Alves, Brian D. Sykes, Larry Fliegel.

The Na⁺/H⁺ exchanger isoform 1 (NHE1) is an integral membrane protein important in the regulation of intracellular pH in the heart by exchanging one intracellular H⁺ for one extracellular Na⁺. It contains an N-terminal transmembrane (TM) domain responsible for ion transport and a C-terminal, cytoplasmic, regulatory domain.

There is currently no high-resolution structure of NHE1; however, a topology model of the protein by Wakabayashi *et al.* (*J. Biol. Chem.* **275**, 7942) suggests that the membrane domain (amino acids 1-500) contains 12 TM helices. We have previously used NMR spectroscopy to determine the structures of peptides representing several TM helices from NHE1. Structures have been determined for TM IV in organic tri-solvent mixture, and for TM helices VII, IX, XI, and VI in DPC micelles.

To provide further insight into the three-dimensional structure of NHE1, we are attempting to express and determine the structures of multiple TM helices. We have recently expressed and purified a two-TM helix segment representing TM VI-VII (amino acids 226-274) of NHE1. These two TM helices have been previously shown to be important in NHE1 function. Furthermore, modelling of NHE1 by Landau *et al.* (*J. Biol. Chem.* **282**, 37854) suggest that these two helices interact and play key roles in ion transport. We are determining the structure of this region in micelles using NMR. NMR spectra of unlabelled TM VI-VII peptide show good quality spectra for structure determination. Further experiments with ¹⁵N labelled peptide will further define the structure. This will provide insight into TM helix interactions in NHE1 and help us to build up the structure of the protein from these TM segments. (Supported by AHFMR, HSF, CIHR, and AIHS).

2081-Pos Board B67

Investigation of the Three-Dimensional Structure of the Caveolin-1 Membrane Interacting Domain

Jinwoo Lee.

The importance of understanding membrane protein structure and function is growing after scientists have recognized that approximately one third of the genome encodes for membrane proteins. Caveolin is an integral membrane protein that is found in high abundance in caveolae. Caveolae are invaginations of the plasma membrane that play an important role in many cellular functions. Interestingly, caveolin plays a dual-role in caveolae. On one hand it is responsible for forming the "flask-like" shape of caveolae, and on the other hand it is intimately involved in cellular processes such as signal transduction and protein trafficking. Alterations in the levels of caveolin protein and mutations of the caveolin protein have been implicated in a variety of disease states including cancer, heart disease, and muscular dystrophy.

Caveolin possesses a unique hairpin-like topology (N- and C- termini are on the same side of the membrane) in the membrane which is thought to facilitate its functions. In particular, mutations of two proline residues (P110A and P132L) in the caveolin-1 transmembrane domain appear to